

Claims:

1. A method of magnifying the signal associated with one or more bases in all or part of the sequence of a target nucleic acid molecule comprising at least the steps of:

a) converting at least a portion of said target sequence to a form suitable for binding an adapter molecule, preferably to single stranded form;

b) binding to at least a portion of said region suitable for binding an adapter molecule, preferably said single stranded region, created in step a) an adapter molecule comprising one or more magnifying tags, or comprising a means for attaching one or more magnifying tags, which tags correspond to one or more bases of said target sequence, preferably corresponding to one or more bases of said region suitable for binding said adapter molecule, preferably said single stranded region, to which said adapter molecule binds or in proximity to said region;

c) optionally ligating said target molecule to said adapter molecule such that at least said magnifying tags remain associated with said target molecule;

d) repeating step a), wherein said region suitable for binding said adapter, preferably said single stranded region, which is created includes one or more bases not associated with a magnifying tag according to step b);

e) repeating steps b) to d) wherein said adapter molecule binds to an adjacent or overlapping region of said target molecule relative to the region to which the adapter molecule of the previous cycle bound wherein said magnifying tags of each cycle of steps a) to c) are ligated together.

2. A method as claimed in claim 1 wherein each magnifying tag corresponds to at least two bases.

3. A method as claimed in claim 1 or 2 wherein said magnifying tags together correspond to at least two bases, preferably at least 4 bases.

5 4. A method as claimed in any one of claims 1 to 3 wherein a chain of magnifying tags are associated with said molecule, preferably comprising 4 or more magnifying tags corresponding to at least 4 contiguous bases.

10 5. A method as claimed in any one of claims 1 to 4 wherein the magnifying tags are nucleic acid sequences of at least 2 bases, preferably 10 to 30 bases in length.

15 6. A method as claimed in any one of claims 1 to 5 wherein said adapter comprises a recognition site for a nuclease with a cleavage site separate from its recognition site.

20 7. A method as claimed in any one of claims 1 to 6 wherein said adapter comprises recognition sites for 2 or more nucleases with cleavage sites separate from their recognition sites, in which cleavage with said
25 nucleases produces single stranded regions which are adjacent or overlapping.

30 8. A method as claimed in any one of claims 1 to 7 wherein more than one adapter is bound in step b), preferably to overlapping or adjacent regions of said portion.

35 9. A method as claimed in claim 8 wherein said adapters bind to overlapping regions of said sequence thereby allowing the association of more than one magnifying tag with each base.

10. A method as claimed in any one of claims 1 to 9 wherein step c) is performed.

11. A method of sequencing all or part of a target nucleic acid molecule wherein the signal associated with each base, or more than one base, is magnified as defined in any one of claims 1 to 10.

12. A method of sequencing as claimed in claim 11 wherein 2 or more bases, preferably 4 or more, are sequenced per cycle of sequencing.

13. A method of sequencing as claimed in claim 11 or 12 wherein the signal associated with each base is magnified by increasing the number of times that said base appears in said sequence.

14. A method of sequencing as claimed in any one of claims 11 to 13 wherein at least a portion of the sequence of said target nucleic acid molecule is magnified as defined in any one of claims 1 to 10, wherein said magnified sequence is optionally converted into readable signals and said sequence is determined by assessment of the signals which are generated.

15. A method as claimed in claim 14 wherein each of said signals comprise a pattern made up of a single signal event which creates a unique signal on each magnifying tag.

16. A method of sequencing all or part of a target nucleic acid molecule comprising at least the steps of:
a) determining the sequence of a portion of said nucleic acid molecule;
b) determining the position of said portion within said nucleic acid molecule; and
c) combining the information obtained in steps a) and b)

to obtain the sequence of said molecule.

17. A method as claimed in claim 16 wherein said
position is determined by reference to a positional
marker.

18. A method as claimed in claim 16 or 17 wherein said
position is determined by reference to a restriction map
of said target molecule.

19. A method of sequencing as claimed in any one of
claims 16 to 18 wherein the portion which is sequenced
has 4 or more bases and/or the position of said portion
within said target molecule is determined with an
accuracy of less than 1 kb.

20. A method as claimed in any one of claims 16 to 19
wherein said portion is sequenced by the method defined
in any one of claims 11 to 15.

21. A method as claimed in any one of claims 16 to 19
wherein said sequence is determined by assessing the
complementarity of a portion of said molecule by a
process comprising at least the steps of:

a) converting at least a portion of said target
sequence to a form suitable for binding a complementary
probe attached to a solid support or carrying a means
for attaching to a solid support, preferably to single
stranded form;

b) binding said complementary probe to at least a
portion, preferably 4 to 12 bases in length, of said
region suitable for binding a complementary probe,
preferably said single stranded region created in step
a);

c) optionally repeating steps a and b) wherein said
complementary probe binds to an adjacent or overlapping
region of said target molecule relative to the region to

which the complementary probe of the previous cycle bound; and

5 d) determining the sequence of said target sequence by identifying the complementary probe(s) to which said target sequence bound.

10 22. A method of sequencing as claimed in any one of claims 16 to 21 wherein a portion of said sequence is determined by a method of sequencing based on magnification as defined in any one of claims 11 to 15 and an adjacent or overlapping portion is determined as described in claim 21.

15 23. A kit for magnifying the one or more bases of a target nucleic acid molecule comprising at least one or more adapters as defined in any one of claims 1 to 10, optionally attached to one or more solid supports.

20 24. A method of magnification or sequencing as claimed in any one of claims 1 to 23 wherein said method is performed on a heterogeneous sample.

25 25. A method of producing a map of a target nucleic acid molecule comprising obtaining sequence information on portions of said sequence by cleavage of said target molecule with one or more nucleases, preferably a nuclease with a cleavage site separate from its recognition site, preferably to produce complementary single stranded regions, and binding of an adapter molecule to a region of said target molecule wherein said adapter molecule carries one or more magnifying tags as described in any one of claims 1 to 9 in which said tag comprises a signalling moiety which corresponds to one or more bases of said region to which said adapter molecule binds and additionally comprises a further signalling moiety which corresponds to a nuclease used for cleavage, wherein said portions

comprise all or part of the cleavage sites of said nucleases and/or all or part of the restriction sites of said nucleases, and determination of the position of said portions within said target sequence.

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AMENDED SHEET